Raman-encoded molecular imaging (REMI) for guiding breast cancer lumpectomy

Yu "Winston" Wang[1], Soyoung Kang[1], Josh D. Doerksen[1], Sara Javid[2], Suzanne Dintzis[3], and Jonathan T.C. Liu[1]*

[1] Department of Mechanical Engineering, University of Washington, Seattle, WA;

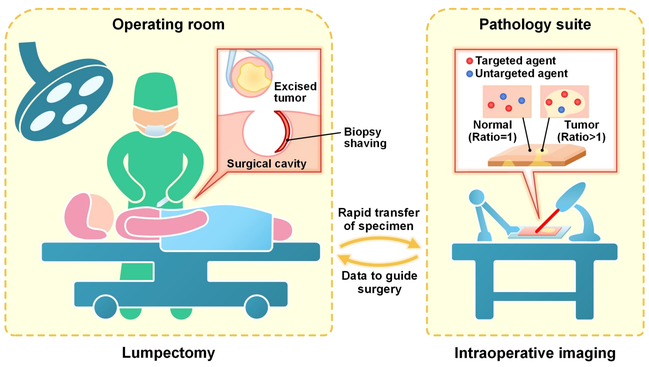
[2] Department of Surgery, University of Washington School of Medicine, Seattle, WA; [3] Department of Pathology, University of Washington School of Medicine, Seattle, WA

❶ Unmet clinical need

Intraoperative guidance of breast cancer lumpectomy

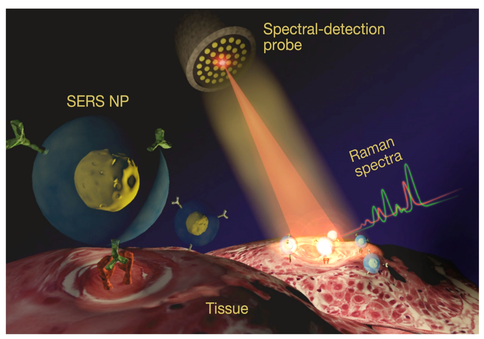
20-60% breast cancer patients require re-excision surgeries when histology (after surgery) reveals positive margins.

In order to guide the complete resection of tumors, intraoperative imaging techniques are needed to identify residual tumor at the surgical margins. A potential means of identifying tumor with **high specificity** is to image disease-related biomarkers at the surgical margin surfaces of freshly excised tissues.



❷ Technical challenge

Molecular biomarkers of disease vary greatly among subjects, between disease subtypes and even within a single subject over time. Therefore, in order to identify tumors with **high sensitivity**, it is necessary to visualize a large multiplexed panel of disease biomarkers.

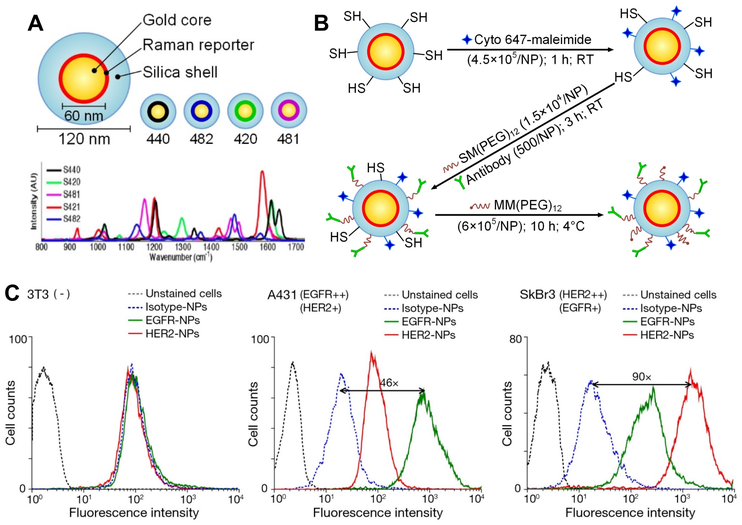


❸ Technical solution: targeted SERS nanoparticles

(A) Surface-enhanced Raman scattering (SERS) nanoparticles (NPs) act as spectral "barcodes" and can enable multiplexed imaging of disease biomarkers if different flavors of NPs are targeted to different biomarkers.

(B) SERS NPs were conjugated to monoclonal antibodies (mAb) to target them against protein biomarkers.

(C) Flow cytometry data reveal a high binding affinity of functionalized NPs to cell-surface biomarkers.



[Wang, Khan, Som, *et al. Technology* (2014)]

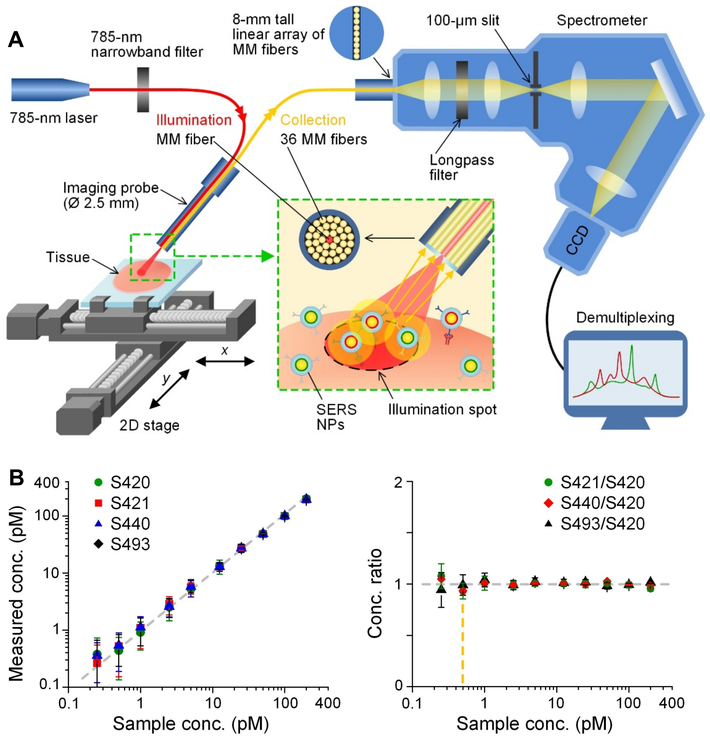
Topical application. In order to avoid toxicity and regulatory complications, SERS NP mixtures are topically applied on the surfaces of freshly excised surgical specimens. Rapid molecular staining is possible in <5 min.

Ratiometric imaging. In order to differentiate between signal that is due to molecular targeting versus nonspecific accumulation, and to accurately quantify relative biomarker expression levels, it is necessary to image the ratio between targeted NPs and an untargeted control NP.

❹ Raman imaging system

(A) Schematic of a spectral-imaging system for the quantification of the concentrations and concentration ratios of SERS NPs applied on tissues.

(B) Linearity of NP detection for a 4-flavor mixture of NPs (1:1:1:1 ratio).



❺ Imaging of human breast tissues to quantify HER2

(A) NP concentrations (n=10) are higher in normal tissues than in tumor tissues due to enhanced nonspecific retention of topically applied NPs.

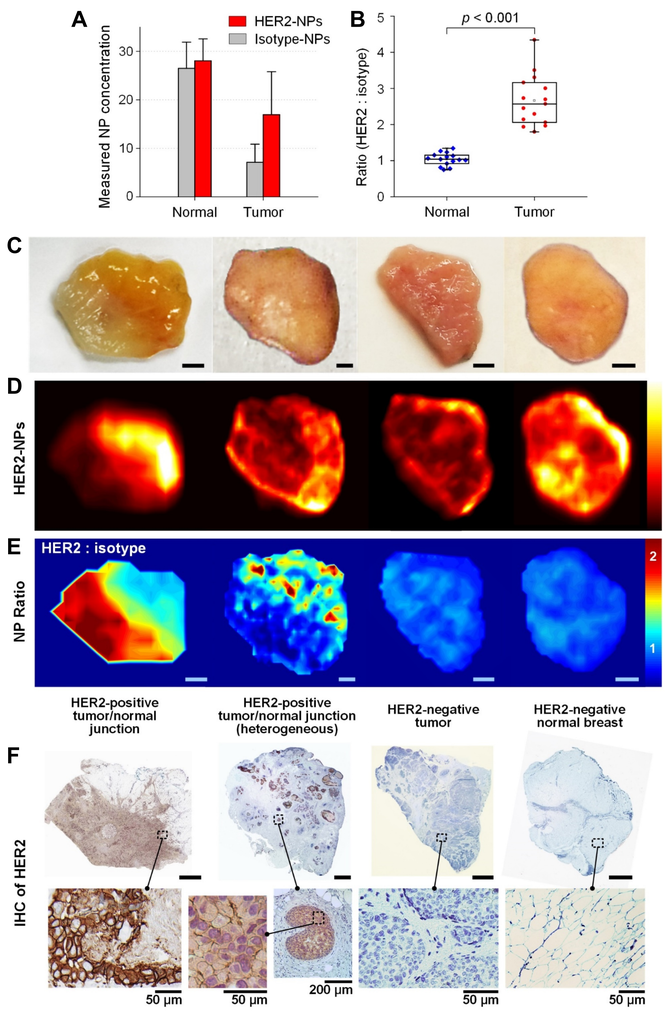
(B) Concentration ratios of HER2-NPs vs. isotype-NPs are elevated in tumor specimens, indicating preferential specific binding of targeted NPs to HER2.

(C) Photographs of tissue specimens from four patients.

(D) Measured concentration of HER2-NPs.

(E) Measured concentration ratio of HER2-NPs vs. isotype-NPs.

(F) Images of IHC staining (HER2) in agreement with ratiometric images.



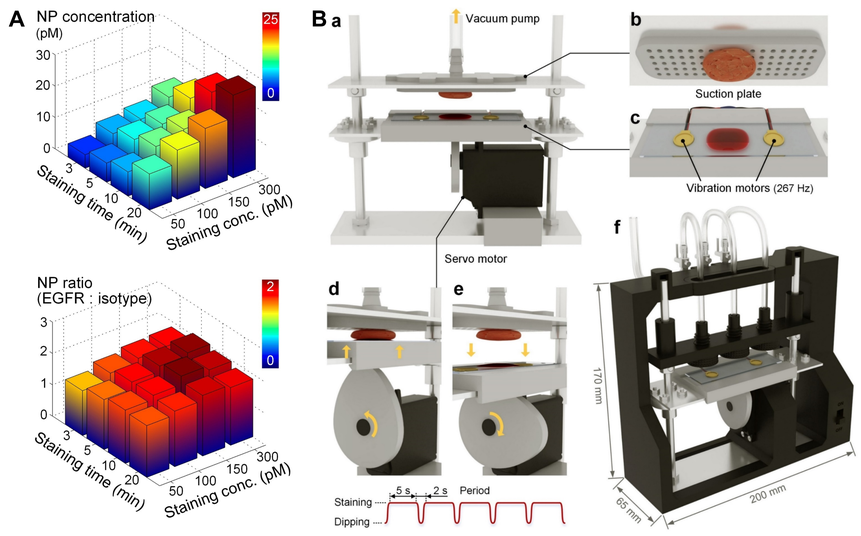
[Wang, Kang, Khan, *et al. Sci Rep* (2016)]

❻ Optimization of topical staining device

(A) NP mixture concentrations and staining durations were varied to optimize the final signal intensity (NP concentration) and molecular contrast (NP ratios).

(B) Automated staining device to enhance the speed and reproducibility of the topical staining procedure. The device is equipped with a suction plate (b) to secure the tissue from above, a servo motor to dip the tissue into and out of a NP mixture (d,e), and high-frequency vibration (c) to enhance NP convection at tissue surfaces and thereby to accelerate binding to biomarker targets.

This dipping and mechanical vibration (DMV) method enables the simultaneous quantification of at least 4 biomarkers on large tissue surfaces after a 2-5 min staining duration with 5 NP flavors (4 targeted and 1 control).



❼ Imaging of human breast tissues to quantify 4 biomarkers

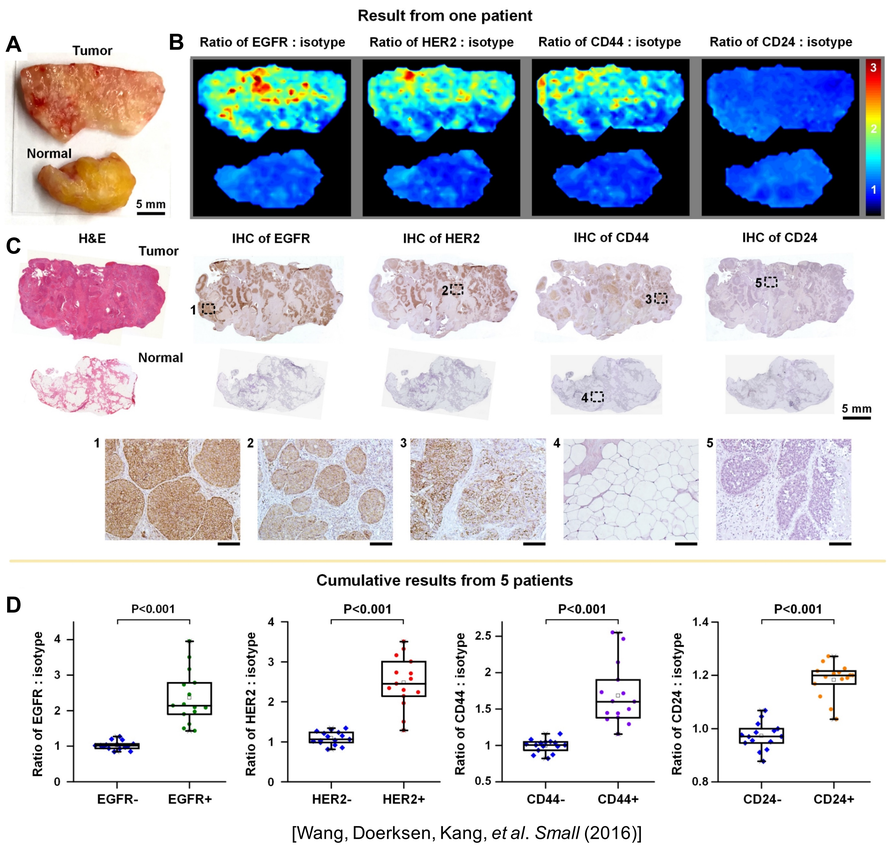
(A) Photograph of a human breast tumor and a normal tissue specimen.

(B) Ratiometric images of EGFR-NPs vs. isotype-NPs, HER2-NPs vs. isotype-NPs, CD44-NPs vs. isotype-NPs and CD24-NPs.

(C) Validation data: H&E and IHC for EGFR, HER2, CD44 and CD24.

(D) Cumulative results with tissues from 5 patients: measured NP ratios on biomarker-negative and biomarker-positive tissue regions.

(E) These results show that the REMI technique is capable of visualizing a large panel of overexpressed tumor biomarkers in 10 min.



[Wang, Doerksen, Kang, *et al. Small* (2016)]

Future work

Clinical translation of this Raman-encoded molecular imaging (REMI) technique to guide breast cancer lumpectomy procedures. Clinical studies will assess the ability to identify residual tumors at the surgical margin surfaces rapidly with high specificity and sensitivity in order to reduce the need for re-excision surgeries.

Acknowledgement: NIH/NIBIB "Pathway to Independence" Award – R00 EB008557 (JTC Liu); NIBIB – R21 EB015016 (JTC Liu).